APPLICATION FOR UNITED STATES LETTERS PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(Case No. 92,678)

Title: PERIPHERALIZATION OF HEMATOPOIETIC STEM CELLS

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BACKGROUND OF THE INVENTION

Field Of The Invention

The invention relates to the manipulation of hematopoietic stem cells. More particularly, the invention relates to methods for increasing the number of hematopoietic stem cells in peripheral blood.

Summary Of The Related Art

Hematopoietic stem cells are primitive, uncommitted progenitor cells that give rise to the lymphoid, myeloid and erythroid lineages of cells in blood. The stem cell population constitutes only a small proportion of the total cells in bone marrow and represents even a far more minuscule proportion of the cells in peripheral blood.

Stem cells have commonly been characterized by their surface antigenic determinants. Tsukamoto et al., U.S. Patent No. 5,061,620 (1991), teaches that a highly stem cell concentrated cell composition is $CD34^+$, $CD10^-$, $CD19^-$ and $CD33^-$. Leon et al., Blood 77:1218-1227 (1991), teaches that about one per cent of $CD34^+$ cells, or about 0.01% of the total marrow cell population, do not express differentiation antigens, such as CD33 (myeloid lineage), CD71 (erythroid lineage) or CD10 and CD5 (lymphoid B and T lineage), and that reduced expression of CD34 expression during maturation is associated with increased expression of the differentiation antigens.

Combinations of antigenic and functional characteristics have also been used to characterize stem cells. Sutherland et al., Proc. Natl. Acad. Sci. USA 87:3584-3588 (1990), teaches that primitive stem cells do not bind to soybean agglutinin, express high levels of CD34, form blast colonies with high plating efficiency and are enriched in long-term culture initiating cells (LTC-IC). Craig et al., Blood Reviews 6:59-67 (1992), teaches that the CFU-GM assay is the most widely used measure of the hematopoietic progenitor viability of a bone marrow or peripheral blood stem cell harvest, and correlates well with per cent $CD34^+$. Spangrude, Immunology Today **10**:344-350 (1989), teaches that stem cells accumulate low levels of rhodamine 123 relative to other bone marrow cell types. Chaudhaury et al., Cell 66:85-94 (1991), teaches that stem cells express high levels of P-glycoprotein relative to other marrow cell types.

The ability to manipulate hematopoietic stem cells has become increasingly important in the development of effective chemotherapeutic and radiotherapeutic approaches to the treatment of cancer. Current approaches to chemotherapy and radiotherapy utilize bone marrow transplantation (BMT). BMT involves removing one to two liters of viable pelvic bone marrow containing stem cells, progenitor cells and more mature blood cells, treating the patient with chemotherapy or radiotherapy to kill tumor cells, and reintroducing bone marrow cells intravenously. BMT, however, suffers from many disadvantages. Harvesting of BM for BMT requires

general anaesthesia, which increases both risk and cost. In addition, if cancer cells are present in the marrow and are not rigorously purged, recurrence of the disease is a distinct risk. Finally, patients whom have undergone pelvic irradiation are not candidates for BMT.

As a result of these difficulties, much interest has been developed in providing methods for obtaining stem cells from peripheral blood for autologous supply of stem cells to patients undergoing chemotherapy. Autologous supply of stem cells from peripheral blood would allow the use of greater doses of chemo- or radiotherapy, but with less risk than BMT. In addition, the use of stem cells from peripheral blood does not require anaesthesia to obtain the stem cells. Also, Lowry, Exp. Hematol. 20:937-942 (1992), teaches that cancer cells in the marrow tend not to peripheralize. The critical limitation in such a procedure, however, lies in the very small number of stem cells ordinarily present in peripheral blood. Lobo et al., Bone Marrow Transplantation 8:389-392 (1991), teaches that addition of peripheral blood stem cells collected in the absence of any peripheralization techniques does not hasten marrow recovery following myeloablative therapy. In contrast, Haas et al., Exp. Hematol. 18:94-98 (1990), demonstrates successful autologous transplantation of peripheral blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF). Thus, increasing the number of stem cells in peripheral

blood by peripheralization techniques is critical to the success of procedures utilizing peripheral blood as a source for autologous stem cell transplantation. Other cytokines may be useful in this regard. Rowe and Rapoport, J. Clin. Pharmacol. 32:486-501 (1992), suggests that in addition to GM-CSF, other cytokines, including macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), erythropoietin, interleukins-1, -2, -3, -4 and -6, and various interferons and tumor necrosis factors have enormous potential.

Another approach to autologous transplantation is to purify stem cells from peripheral blood using immunoaffinity techniques. These techniques hold promise not only for autologous stem cell transplantation in conjunction with chemotherapy, but also for gene therapy, in which purified stem cells are necessary for genetic manipulation to correct defective gene function, then reintroduced into the patient to supply the missing function. However, Edgington, Biotechnology 10:1099-1106 (1992), teaches that current procedures require three separate four hour sessions to process enough cells in the absence of peripheralization. DePalma, Genetic Engineering News, Vol. 12, May 1, 1992, teaches that this can be improved by treatment with G-CSF for peripheralization.

These studies underscore the importance of developing new methods to effect the peripheralization of hematopoietic stem cells. One possibility is to search for new ways to release stem cells from the bone marrow environment into the periphery.

Unfortunately, little is known about the types of molecular interactions that hold hematopoietic stem cells in the marrow environment <u>in vivo</u>. Recently, some <u>in vitro</u> studies have been undertaken to look at the role of integrins, fibronectin, and other surface antigens in binding between stem cells and bone marrow stromal cells.

Integrins are a large family of integral membrane glycoproteins having over 16 heterodimeric members that mediate interactions between cells, interactions between cells and the extracellular matrix, and interactions involved in embryonic development and regulation of T-cell responses. Among integrins, the VLA-5 ($\alpha^5\beta_1$) complex is widely distributed and functions as a receptor for fibronectin. The VLA-4 ($\alpha^4\beta_1$) complex is expressed at substantial levels on normal peripheral blood B and T cells, thymocytes, monocytes, and some melanoma cells as well as on marrow blast cells and erythroblasts. Ligands for VLA-4 are vascular cell adhesion molecule-1(VCAM-1) and CS-1, an alternately spliced domain within the Hep II region of fibronectin. Another group of integrins (CDIIa/CD18, CDIIb/CD18, and CDIIc/CD18) share the common β_2 chain and are variably expressed on peripheral T cells, monocytes, and mature granulocytes. Ligands for β_2-integrins include members of the Ig superfamily (ICAM-1 and ICAM-2) found on activated endothelial cells.

Teixido et al., J. Clin. Invest. <u>90</u>:358-367 (1992), teaches that in an <u>in vitro</u> model, interactions between VLA-4/VCAM-1, VLA-5/fibronectin and β_2-integrin/ICAM-1 are all three important for

adhesion between bone marrow stromal cells and cells expressing high levels of CD34. Simmons et al., Blood 80: 388-395 (1992), teaches that in an *in vitro* model, adhesion between stromal cells and $CD34^+$ cells was predominantly dependent on the VLA-4/VCAM-1 interaction and was largely inhibited by monoclonal antibodies to either VLA-4 or VCAM-1, with fibronectin playing a minor role in binding. Williams et al., Nature 352:438-441 (1991), using *in vivo* mouse studies, teaches that adhesion of murine hematopoietic stem cells to stromal cell ECM is partly promoted by proteolytic fragments of fibronectin containing an alternatively spliced region of the IIICS domain, and suggests that the interaction is likely to be mediated by VLA-4. All of these studies utilized antibodies to prevent adherence between stem cells and their microenvironment. However, none have analyzed whether such interactions are reversible, or perturbable after adherence has taken place. These results indicate the need for further studies to determine what interactions between the bone marrow environment and hematopoietic stem cells are responsible for keeping the stem cells within that environment *in vivo* and whether such interactions can be perturbed to effect peripheralization of stem cells.

There is, therefore, a need for new methods for peripheralizing stem cells, both for scientific investigatory purposes for understanding the processes of peripheralization and homing, and for the development of better methods of peripheralization for autologous stem cell transplantation in the course of cancer treatment or gene therapy. Preferably, such

methods should produce even higher levels of stem cells in peripheral blood than existing methods provide.

BRIEF SUMMARY OF THE INVENTION

In a first aspect, the invention provides a novel method for increasing the number of hematopoietic stem cells and $CD34^+$ cells in peripheral blood, which is also known as "peripheralization" or "mobilization" of hematopoietic stem cells and $CD34^+$ cells. This method comprises the step of blocking VLA-4 antigens on the surface of hematopoietic stem cells and $CD34^+$ cells. Various agents can be used to mediate such blocking, including anti-VLA-4 antibodies which may optionally be humanized or chimeric, Fab, Fab', $F(ab')_2$ or F(v) fragments thereof, heavy or light chain monomers or dimers thereof, or intermixtures of the same, soluble fibronectin or fibronectin peptides containing the amino acid sequence EILDV or conservatively substituted amino acid sequences, or soluble VCAM-1 or VCAM-1 peptides.

In another aspect, the invention provides a novel method for peripheralizing hematopoietic stem cells and $CD34^+$ cells with greater effectiveness than cytokine treatment alone provides. According to this aspect of the invention, the method comprises blocking VLA-4 antigens on the surface of hematopoietic stem cells and $CD34^+$ cells, as in the first aspect of the invention, in combination with stimulating hematopoietic stem cells to proliferate. The step of stimulating hematopoietic stem cells to proliferate can be carried out by using a cytokine, preferably G-CSF, stem cell factor or GM-CSF, but alternatively M-CSF, erythropoietin, interleukins-1, -2, -3, -4, -6, or 11.

It is an object of the invention to provide a method for peripheralizing hematopoietic stem cells and $CD34^+$ cells as an experimental model for investigating hematopoiesis, homing of stem cells to the bone marrow, and cytokine-induced peripheralization of stem cells. It is a further object of the invention to provide a method for optimizing peripheralization of hematopoietic stem cells and $CD34^+$ cells to provide stem cell-enriched peripheral blood for autologous transplantation following chemo- or radiotherapy. It is a further object of the invention to provide a method for peripheralizing hematopoietic stem cells to maximize the yield of the purified of hematopoietic stem cells from peripheral blood, either for autologous transplantation of the stem cells following chemo- or radiotherapy, or for use in gene therapy. It is a further object of the invention to provide a method for peripheralizing stem cells and $CD34^+$ cells without risk of causing cytokine-induced cell differentiation of normal stem cells or proliferation of contaminating leukemia cells. It is a further object of the invention to provide a peripheralization technique that has predictable timing for the peak of progenitor content in peripheral blood for scheduling leukapheresis.

The invention satisfies each of these objects by providing a method for peripheralizing stem cells and $CD34^+$ cells by blocking VLA-4 antigen on the surface of hematopoietic stem cells. This effect can be increased by the use of such blocking in conjunction with approaches to amplify stem cells to produce a synergistic effect.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows profiles of both total white blood cells and CFU in peripheral blood before and after treatment of macaques (panels A and C) or a baboon (panel B) with anti-VLA-4 antibodies (monoclonal antibody HP1/2). Dashed lines represent total white blood cell counts, as recorded on the right vertical axes. Cross-hatched boxes represent CFU-GM, as recorded in the left vertical axes. Black boxes represent BFUe, as represented on the left vertical axes. Downward-pointing arrows represent points of administration of antibody. Horizontal axes represent days before and after first administration of anti-VLA-4 antibody.

Figure 2 shows profiles of both total white blood cells and CFU in peripheral blood before and after treatment of an animal with the anti-CD18 monoclonal antibody Ab60.3. All symbols are as in Figure 1.

Figure 3 shows results of combined treatment with G-CSF and anti-VLA-4 monoclonal antibody HP1/2. In panel A, symbols are as in Figure 1, except that narrow downward-pointing arrows represent points of G-CSF administration, bold downward-pointing arrows represent points of antibody administration, and dotted lines (with triangles) represent total lymphocyte counts. In panel B, the same symbols show the results for a control animal treated with GCSF alone.

Figure 4 shows high proliferative potential (HPP) progenitors (colonies over 0.5 mm in diameter, having a compact center) resulting from combined treatment with GCSF and HP 1/2 antibody (panel A) or GCSF alone (panel B). Symbols are as in Figure 3.

Figure 5 shows the nucleotide sequences encoding the variable regions of the heavy and light chains of anti-VLA-4 monoclonal antibody HP 1/2. Panel A is the nucleotide sequence encoding the variable heavy region, with the first nucleotide representing the beginning of the first codon. Panel B is the nucleotide sequence encoding the variable light region, with the first nucleotide representing the beginning of the first codon.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to the manipulation of hematopoietic stem cells. More particularly, the invention relates to the peripheralization of hematopoietic stem cells and other $CD34^+$ cells.

In a first aspect, the invention provides a method for peripheralizing hematopoietic stem cells and $CD34^+$ cells, comprising the step of blocking VLA-4 antigens on the surface of hematopoietic stem cells and $CD34^+$ cells. For purposes of the invention, the term "blocking VLA-4 antigens" is intended to mean interfering with interactions between VLA-4 antigens and either VCAM-1 or fibronectin on the surface of stromal cells or in the extracellular matrix (ECM). As demonstrated herein, such blocking of VLA-4 antigens causes peripheralization of stem cells and $CD34^+$ cells. This demonstration utilized a monoclonal antibody against VLA-4 as a blocking agent. Those skilled in the art will recognize that, given ths demonstration, any agent that can block VLA-4 antigens can be successfully used in the method of the invention. Thus, for purposes of the invention, any agent capable of blocking VLA-4 antigens on the surface of hematopoietic stem cells is considered to be an equivalent of the monoclonal antibody used in the examples herein. For example, the invention contemplates as equivalents at least peptides, peptide mimetics, carbohydrates and small molecules capable of blocking VLA-4 antigens on the surface of $CD34^+$ cells or hematopoietic stem cells.

In a preferred embodiment, the blocking agent that is used in the method of the invention to block VLA-4 antigens on the surface of hematopoietic stem cells and $CD34^{+}$ cells is a monoclonal antibody or antibody derivative. Preferred antibody derivatives include humanized antibodies, chimeric antibodies, Fab, Fab', $F(ab')_2$ and F(v) antibody fragments, and monomers or dimers of antibody heavy or light chains or intermixtures thereof. The successful use of monoclonal antibody OKT3 to control allograft rejection indicates that murine monoclonal antibodies can be effective in therapeutic applications. Some patients have been able to receive only one or two doses of OKT3 before developing a human anti-mouse antibody (HAMA) response. This should not represent a significant problem for use of the method of the invention in therapeutic applications, since such applications in either anti-cancer or gene therapy are unlikely to be highly repeated. Thus, monoclonal antibodies against VLA-4 are a preferred blocking agent in the method according to the invention. Human monoclonal antibodies against VLA-4 are another preferred blocking agent in the method according to the invention. These can be prepared using in vitro-primed human splenocytes, as described by Boerner et al., J. Immunol. 147:86-95 (1991). Alternatively, they can be prepared by repertoire cloning as described by Persson et al., Proc. Natl. Acad. Sci. USA 882432-2436 (1991) or by Huang and Stollar, J. of Immunol. Methods 141:227-236 (1991). Another preferred blocking agent in the method of the invention is a chimeric antibody having anti-VLA-4 specificity and a human

antibody constant region. These preferred blocking agents can be prepared according to art-recognized techniques, as exemplified in U.S. Patent No. 4,816,397 and in Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984). Yet another preferred blocking agent in the method of the invention is a humanized antibody having anti-VLA-4 specificity. Humanized antibodies can be prepared according to art-recognized techniques, as exemplified in Jones et al., Nature 321:522 (1986); Riechmann, Nature 332:323 (1988); Queen et al., Proc. Natl. Acad. Sci. USA 86:10029 (1989); and Orlandi et al., Proc. Natl. Acad. Sci. USA 86:3833 (1989). Those skilled in the art will be able to produce all of these preferred blocking agents, based upon the nucleotide sequence encoding the heavy and light chain variable regions of HP1/2 [SEQ. ID. NOS. 1 and 2], as shown in Figure 5, using only well known methods of cloning, mutagenesis and expression (for expression of antibodies, see e.g., Boss et al., U.S. Patent No. 4,923,805). Those skilled in the art will recognize that any of the above-identified antibody or antibody derivative blocking agents can also act in the method of the invention by binding the receptor for VLA-4, thus blocking the VLA-4 antigen on the surface of hematopoietic stem cells, within the meaning of this term for purposes of the invention. Thus, antibody and antibody derivative blocking agents according to the invention include embodiments having binding specificity for VCAM-1 or fibronectin, since these molecules appear to either be important in the adhesion between stem cells and stromal cells or the extracellular matrix or interfere with traffic of stem cels through

other tissues and blood.

In another preferred embodiment, the blocking agents used in the method according to the invention are not antibodies or antibody derivatives, but rather are soluble forms of the natural binding proteins for VLA-4. These blocking agents include soluble VCAM-1 or VCAM-1 peptides as well as fibronectin, fibronectin having an alternatively spliced non-type III connecting segment and fibronectin peptides containing the amino acid sequence EILDV or a similar conservatively substituted amino acid sequence. These blocking agents will act by competing with the stromal cell- or ECM-bound binding protein for VLA-4 on the surface of stem cells.

In this method according to the first aspect of the invention, blocking agents are preferably administered parenterally. The blocking agents are preferably administered as a sterile pharmaceutical composition containing a pharmaceutically acceptable carrier, which may be any of the numerous well known carriers, such as water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, or combinations thereof. Preferably, the blocking agent, if an antibody or antibody derivative, will be administered at a dose between about 0.1 mg/kg body weight/day and about 10 mg/kg body weight/day. For non-antibody or antibody derivative blocking agents, the dose range should preferably be between molar equivalent amounts to these amounts of antibody. Optimization of dosages can be determined by administration of the blocking agents, followed by CFU-GM assay of peripheral blood. The preferred dosage should produce an increase of at least 10-fold in

the CFU-GM counts in peripheral blood.

In a second aspect, the invention provides a method for peripheralizing hematopoietic stem cells that is far more effective than cytokine treatment alone. According to this aspect of the invention, the method comprises the step of blocking VLA-4 antigens on the surface of hematopoietic stem cells in combination with the step of stimulating the hematopoietic stem cells to proliferate in vivo. The step of blocking VLA-4 antigens on the surface of hematopoietic stem cells is carried out in exactly the same fashion that is described for the first apsect of the invention. The step of stimulating the hematopoietic stem cells to proliferate in vivo is preferably carried out through the administration of cytokines.

Preferred cytokines for stimulating hematopoietic stem cells to proliferate include granulocyte colony-stimulating factor (G-CSF), stem cell factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), erythropoietin, interleukin-1, -2, -3, -4, -6, and -11. Most preferred are G-CSF, stem cell factor and GM-CSF, because all three of these are known to cause proliferation of stem cells. The ability of G-CSF and GM-CSF to stimulate proliferation of progenitors is well established (see, e.g., Metcalf, Nature 339:27-30 (1989)), as is their ability to cause peripheralization of hematopoietic stem cells (see, e.g., Haas et al., Exp. Hematol. 18:94-98 (1990) and Blood 72:2074 (1988). This ability has also been established for stem cell factor (Andrews et al., Blood

80:920-927 (1992)). In addition, the enormous potential of these other cytokines identified herein has been recognized (see Rowe and Rapoport, J. Clin. Pharmacol. 32:486-501 (1992)). For purposes of the invention, stimulation of hematopoietic stem cells to proliferate can be carried out by any cytokine that is capable of mediating such proliferation in vivo. Thus, for purposes of the invention, any cytokine that can stimulate hematopoietic stem cells to proliferate in vivo is considered to be equivalent to G-CSF, stem cell factor and GM-CSF, which are also considered to be equivalent to each other.

In this method according to the second aspect of the invention, cytokines are preferably administered parenterally. The cytokines are preferably administered as a sterile pharmaceutical composition containing a pharmaceutically acceptable carrier, which may be any of the numerous well known carriers, such as water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, or combinations thereof. Preferably, the cytokine, if G-CSF, will be administered at a dose between about 1 μg/kg body weight/day and about 50 μg/kg body weight/day, most preferably at about 10-15 μg/kg body weight/day. Most preferably, cytokines will be administered over a course of from about four to about ten days. Optimization of dosages can be determined by administration of the cytokine and administration of the blocking agents, followed by CFU-GM assay of peripheral blood. The preferred dosage should produce an increase of at least 5-fold in the CFU-GM counts per milliliter of peripheral blood, compared with cytokines alone.

According to this aspect of the invention, the step of blocking of VLA-4 antigens on the surface of hematopoietic stem cells or $CD34^+$ cells and the step of stimulating these cells to proliferate can be carried out concomitantly or sequentially. In a preferred embodiment, the steps are carried out sequentially, preferably stimulating the $CD34^+$ or hematopoietic stem cells to proliferate being the first step.

The instant invention is useful for many purposes. The methods of peripheralizing hematopoietic stem cells or $CD34^+$ cells is of value in scientific research dedicated to understanding the molecular interactions and molecular signals involved in the homing of these cells to bone marrow, as well as their trafficking in response to certain infections and trauma. The invention also provides sources of peripheral blood that is enriched in $CD34^+$ and hematopoietic stem cells, thus making the methods of the invention useful for therapeutic applications involving autologous transplantation of these cell types following chemotherapy or radiotherapy or in the course of gene therapy. The invention provides many advantages over current exclusively cytokine-based techniques. For example, peripheralization can be obtained without risk of cytokine-induced cell differentiation of normal cells or proliferation of contaminating leukemia cells. In addition, in the method of the invention, the timing of the peak of progenitors in peripheral blood is consistently between about 24 and about 72 hours from first injection of antibody, thus making the most

beneficial timing for leukapheresis more predictable.

The efficacy of specific embodiments of methods according to both aspects of the invention is demonstrated in the examples. According to the first aspect of the invention, monoclonal antibodies against VLA-4 were administered to both macaques and a baboon. These antibodies, mouse monoclonal HP1/2, have previously been described by Sanchez-Madrid, J. Biol. Chem. 266:10241 (1991), and are known to block VLA-4 antigen on various cell surfaces. In the present case, administration of these antibodies resulted in as much as a 80-fold increase (average of 40-fold) in CFU-GM present in peripheral blood. The well known CFU-GM assay is the most widely used measure of the hematopoietic progenitor viability of a PBSC harvest and correlates well with per cent $CD34^+$ cells present in peripheral blood (see Craig et al., Blood Reviews 6:59-67 (1992)). Thus, these results demonstrate that, in a primate, blocking VLA-4 antigen on the surface of hematopoietic stem cells and $CD34^+$ cells results in peripheralization of the hematopoietic stem cells and $CD34^+$ cells. These results should be applicable to humans as well.

According to the second aspect of the invention, monoclonal antibodies against VLA-4 were administered to a macaque after five days of treatment with G-CSF. It is well known that G-CSF can stimulate hematopoietic stem cells and $CD34^+$ cells in vivo (see Metcalf, Nature 339:27-30 (1989)). G-CSF alone caused an increase in CFU-GM present in peripheral blood by days 4 and 5 of treatment. After discontinuation of G-CSF treatment and commencement of

treatment with anti-VLA-4 antibodies, the number of CFU-GM in peripheral blood increased even more dramatically. It is well established that G-CSF alone does not cause the type of post-treatment increases in CFU-GM that were observed in the present case and a control experiment using G-CSF alone confirmed this. Thus, these results demonstrate that, in a primate, blocking VLA-4 antigen on the surface of hematopoietic stem cells and $CD34^+$ cells in combination with stimulating these cells to proliferate has a synergistic effect. There is no reason to believe that these results will not apply equally well to humans.

Although not wishing to be bound by theory, Applicant believes that blocking VLA-4 antigens on the surface of hematopoietic stem cells and $CD34^+$ cells causes peripheralization of these cells by mediating release of the cells from the marrow environment via disruption of interactions between VLA-4 and its microenvironmental ligands, such as fibronectin and/or VCAM-1 on stromal cells or in the ECM. Stimulating hematopoietic stem cells and $CD34^+$ cells to proliferate is believed to cause peripheralization at least in part via sheer increase in the numbers of these cells. Thus, it is believed that blocking VLA-4 antigens in combination with stimulating proliferation effect peripheralization by complementary mechanisms. The observed synergisitic effect between anti-VLA-4 antibodies and G-CSF supports this interpretation. Since these mechanisms appear to be complementary, the observed synergistic effect should be obtained, regardless of whether blocking of VLA-4 antigens and stimulation of proliferation are carried out

concomitantly or in sequence.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not intended to be limiting in nature.

Example 1
Peripheralization Of Stem Cells Using An Anti-VLA-4 Antibody

Three macaques and one baboon were injected intravenously with anti-VLA-4 mouse monoclonal antibody HP1/2 (1 mg/kg body weight/day) for four consecutive days. At various time points during and after completion of treatment, peripheral blood was collected and mononuclear cells were collected using a conventional Ficoll-Hypaque separation procedure. Total white blood cells were calculated from the number of mononuclear cells recovered per milliliter of blood. CFU-GM and BFUe were determined according to conventional assays (see, e.g., Papayannopoulou et al., Science 224:617 (1984)). The results of these studies are shown for two macaques (panels A and C) and one baboon (panel B) in Figure 1. These results demonstrate that treatment of these primates with an anti-VLA-4 monoclonal antibody causes a small increase (up to 2-fold) in the total white blood cell count, peaking at about 2 to 4 days after beginning of treatment. More importantly, the total CFU-GM per ml blood increased much more dramatically (about 40-fold), also peaking at about 2 to 4 days after beginning of treatment. In another macaque, a CFU-GM increase of about 8-fold

was observed after a single injection of antibody (data not shown). Given the well established use of the CFU-GM assay to measure the repopulating potential of hematopoietic progenitors and the correlation between CFU-GM and percentage $CD34^+$, these results establish that the anti-VLA-4 antibodies cause peripheralization of stem cells.

As a control experiment, another macaque was treated with a monoclonal antibody against CD18, which is also present on stem cells and has been proposed to be important in interactions involving stem cells. Antibody was delivered by intravenous injection for three days at a dosage of 2mg/kg of body weight/day. The results of this control experiment are shown in Figure 2. Total white blood cell counts did increase with this treatment, consistent with previous experiments with rabbits. However, total GFU-GM showed no increase after treatment with anti-CD18 monoclonal antibodies. These results confirm that the peripheralization of stem cells observed upon treatment with anti-VLA-4 monoclonal antibody was indeed due to specific blocking of VLA-4.

Example 2
Synergistic Peripheralization Of Stem Cells Resulting From Treatment With Both Anti-VLA-4 Antibody In Combination With G-CSF

A baboon was treated with recombinant human G-CSF twice daily for five consecutive days. Each G-CSF treatment consisted of intravenous injection of 15 micrograms G-CSF per kilogram of body weight. After the five days of G-CSF administration, the baboon

received two injections, spaced one day apart, of anti-VLA-4 monoclonal antibody (HP1/2). Each injection contained 1 milligram antibody per kilogram body weight. Total white blood cells and CFU-GM were determined as described in Example 1. The results are shown in Figure 3. As shown in panel A of that figure, G-CSF resulted in the expected increase in CFU-GM by days 4 and 5 of treatment, along with a marked increase in total white blood cells. Surprisingly, after the administration of anti-VLA-4 antibody beginning after the last day of a 5 day G-CSF treatment, yet another marked increase in CFU-GM was observed, this time without any increase in total white blood cells. This second increase resulted in about a six-fold improvement in the number of CFU-GM, relative to G-CSF alone. A control animal treated with G-CSF alone according to the same protocol showed a continuous decline in peripheral blood CFU after cessation of treatment (see figure 3, panel B). These results indicate that treatment with anti-VLA-4 antibody was responsible for this second increase in CFU-GM. Thus, combined treatment with anti-VLA-4 antibody and G-CSF results in a synergistic effect, causing far greater increases in CFU-GM than treatment by either G-CSF or anti-VLA-4 antibodies alone.

Example 3
Analysis Of High Proliferative Potential Cells In Peripheral Blood Following Combined Treatment With G-CSF And Anti-VLA-4 Antibody.

In the experiments described in Example 3, high proliferative potential (HPP) cells were also counted. HPP cells are cells that

give rise to colonies that are macroscopically visible, over 0.5 mm in diameter with a dense center on the analysis grid. Presence of these cells is associated with greater repopulation capacity and such cells are believed to be earlier progenitors. The results are shown in Fig. 4. The observed disparity in peripheral blood HPP cells between G-CSF treatment alone and G-CSF treatment in combination with anti-VLA-4 antibodies is even greater than the disparity observed for CFU-GM. These results suggest that the combined treatment not only produces more progenitors, but also produces earlier progenitors having potentially greater repopulation capacity.